Docket No.: 211110US0DIV

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Yasuhiro MIHARA et al. : ATTN: APPLICATION DIVISION

SERIAL NO: NEW APPLICATION :

FILED: HEREWITH :

FOR: NOVEL MICROORGANISMS AND METHOD
FOR PRODUCING XYLITOL OR D-XYLULOSE

PRELIMINARY AMENDMENT, SEQUENCE LISTING, AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as shown in the marked-up copy following this amendment.

Page 1, before line 1, please insert

--This application is a Divisional of U.S. Application Serial No. 09/347,001, filed on July 2, 1999, now pending.--

Page 17, replace the paragraph starting on line 3 and ending on line 20 with the following paragraph:

--The multiple sequence alignment and evolution distance calculation can be performed by, for example, using a commercially available software such as CLUSTAL W

included in the software collection "Phylogeny Programs". These programs are publically available from the Department of Genetics, University of Washington, Seattle, WA, USA at their website: evolution.genetics.washington.edu/phylip/software.html. See also Thompson, D. J., et al. Nucleic Acids Res. 22, 4673-4680 (1994).

The phylogenetic tree can be prepared also by a generally available software (e.g., Tree View, Tree drawing software for Apple Macintosh: by Roderic, D., Page 1995, Institute of Biomedical and Life Sciences, University of Glasgow, UK). Specifically, results obtained by computation on CLUSTAL W can be output as PHLYP format data, and they can be processed by Tree View. PHLYP (Felsenstein, J. (1995) Phylogenetic inference package, version 3.5.7., Department of Genetics, University of Washington, Seattle, WA, USA) is also included in the aforementioned Phylogeny Programs.--

Page 26, replace the paragraph beginning at line 4 with the following paragraph:

--Then, about 3000 strains of osmophilic bacteria obtained as described above were cultured in a medium containing 20% (w/v) D-glucose, 0.1% urea, and 0.5% yeast extract at 30°C for 5 days, and the medium was analyzed by HPLC to screen for a strain having the xylitol or D-xylulose producing ability. As a result, five bacterial strains separated from soil collected from the back of Tama river, Kawasaki-shi, Kanagawa-ken, were found to have the ability to produce xylitol from glucose. These strains were each designated as strains P528, S877, S1009, S1019 and S1023. These five strains were assigned private numbers of AJ14757, AJ14758, AJ14759, AJ14760, and AJ14761 in this order, and have been deposited since June 18, 1998 at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (zip code: 305-8566, 1-3 Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, Japan), as deposition numbers of FERM P-16848, FERM P-16849, FERM P-16850, FERM P-16851, and FERM P-16852 in this order, and transferred from the original

deposition to international deposition based on Budapest Treaty on June 14, 1999, and has been deposited as deposition numbers of FERM BP-6751, FERM BP-6752, FERM BP-6753, FERM BP-6754, and FERM BP-6755.--

Please delete the original Sequence Listing at pages 39-42 without prejudice.

Page 46 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.

IN THE CLAIMS

Please cancel Claims 1-15.

Please add the following new claims.

--16. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium having an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose in the medium, and

collecting xylitol or D-xylulose from the medium,

wherein the bacterium belongs to the family *Acetobacteracea*, which is located between *Acetobacter methanolicus* and *Acetobacter pasteurianus* as determined by comparison of the 16S RNA gene nucleotide sequence of said strain with the 16S rRNA gene nucleotide sequences of *Acetobacter methanolicus* and *Acetobacter pasteurianus* using molecular taxonomic analysis.

17. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium having an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose in the medium, and

collecting xylitol or D-xylulose from the medium,

wherein the bacterium has the following characteristics:

(a) an ability to produce xylitol or D-xylulose from glucose;

(b) quinone type: ubiquinone-10;

(c) GC content of DNA: about 56 to 57%;

(d) a weak ability to produce acetic acid from ethanol; and

(e) grows in the presence of 30% glucose.

18. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium belonging to the genus *Asaia* which has an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose in the medium, and

collecting xylitol or D-xylulose from the medium.

19. (New) The method according to Claim 18, wherein the bacterium belongs to *Asaia ethanolifaciens*.

20. (New) The method according to Claim 19, wherein the bacterium has a 16S rRNA gene comprising the nucleotide sequence of SEQ ID NO: 1.

21. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium having an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose in the medium, and

collecting xylitol or D-xylulose from the medium,

wherein the bacterium belongs to the family *Acetobacteracea,* which is located between *Gluconobacter oxydans* subsp. *Oxydans* and *Acetobacter aceti* as determined by comparison of the 16S rRNA gene nucleotide sequences of *Gluconbacter oxydans* subsp. *oxydans* and *Acetobacter aceti* using molecular taxonomic analysis.

22. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium having an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose in the medium, and

collecting xylitol or D-xylulose from the medium,

wherein the bacterium an isolated microbial strain belonging to the family *Acetobacteracea*, which has the following characteristics:

(a) an ability to produce xylitol or D-xylulose from glucose;

(b) quinone type: ubiquinone-10;

(c) GC content of DNA: about 52 to 53%;

(d) a weak ability to produce acetic acid from ethanol; and

(e) grows in the presence of 30% glucose.

23. (New) A method for producing xylitol or D-xylulose, which comprises:

culturing a bacterium belonging to the genus *zucharibacter* which has an ability to produce xylitol or D-xylulose from glucose in a suitable medium to accumulate xylitol or D-xylulose from the medium, and

collecting xylitol or D-xylulose from the medium.

24. (New) The method according to Claim 23, wherein the bacterium belongs to *Zucharibacter floricola*.

25. (New) The method according to Claim 24, wherein the bacterium has a 16S rRNA gene comprising the nucleotide sequence of any one of SEQ ID Nos: 2, 3, 4 or 5.--

SUPPORT FOR THE AMENDMENTS

Page 1 of the application has been amended to recite the continuing application data. Page 17 has been amended to make the same changes as submitted in the response filed on February 05, 2001 in the parent application. Page 26 has been amended to recite the date of deposit. Newly added Claims 16-25 are supported by the specification. The substitute Sequence Listing is supported by the original Sequence Listing at pages 39-42. No new matter is believed to have been added to this application by these amendments.

REMARKS

Claims 16-25 are pending in this application.

This application is a Divisional of U.S. Application Serial No. 09/347,001, filed on July 2, 1999, now pending.

Applicant submits that the sequence information recorded in the computer-readable Sequence Listing filed May 24, 2000 in the parent application, is identical to the paper copy of the Sequence Listing attached herewith.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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ATTORNEY DOCKET NO.: 211110US0DIV
SERIAL NO.: New Application

MARKED-UP COPY

Serial No.: 211110US0DIV
Amendment Filed On: July 12, 2001

IN THE SPECIFICATION

Please amend the specification as follows.

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IN THE CLAIMS

Claims 1-15 deleted.

Claims

--16. (New)

17. (New)

18. (New)

19. (New)

20. (New)

21. (New)

22. (New)

23. (New)

24. (New)

25. (New)--